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(54) Title: A TGF-BETA SUPERFAMILY TYPE II RECEPTOR HAVING BINDING AFFINITY

(57) Abstract

The present invention relates to a novel TGF-β superfamily type II receptor having binding affinity for a bone morphogenic protein, osteogenic protein 1 (OP-1). The novel type II receptor has a serine/threonine kinase domain followed by a long serine and threonine rich region in the C-terminal end. The invention further provides nucleic acids encoding the novel type II receptor as well as vectors and host cells expressing the nucleic acids and recombinant production methods using the nucleic acids. Complexes of the novel type II receptor with an appropriate TGF-β superfamily type I receptor are also provided.

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A TGF-BETA SUPERFAMILY TYPE II RECEPTOR HAVING BINDING AFFINITY

Field of the Invention

The present invention generally relates to a novel TGF-β superfamily type II receptor, particularly to a receptor for bone morphogenic protein, corresponding nucleic acid molecules and their use.

Background of the Invention

The transforming growth factor-β (TGF-β) superfamily consists of a family of structurally-related proteins, including three different mammalian isoforms of TGF-β (TGF-β1, β2, and β3), activins, inhibins, müllerian-inhibiting substance and bone morphogenic proteins. For reviews of these TGF-β proteins, see Roberts & Sporn, Peptide Growth Factors and Their Receptors, Pt.1, pp. 419-472 (Berlin:Springer-Verlag, 1990); and Moses et al., Cell 63:245-247 (1990). Glial cell line derived neurotrophic factor (GDNF) is also a member of the TGF-β superfamily (see Lin et al., Science 260:1130-1132 (1993)). The proteins of the TGF-β superfamily have a wide variety of biological activities. TGF-β acts as a growth inhibitor for many cell types and appears to play a central role in the regulation of embryonic development, tissue regeneration, immuno-regulation, as well as in fibrosis and carcinogenesis (Roberts & Sporn, supra.)

TGF-ßs and activins transduce their signals through the formation of heteromeric complexes of two different types of serine/threonine kinase receptors, i.e. type I receptors of about 50-55 kDa and type II receptors of about 70-80 kDa (Massagué et al., Trends Cell Biol. 4, 172-178, 1994). Type II receptors bind ligands in the absence of type I receptors, but they require their respective type I receptors for signaling, whereas type I receptors require their respective type II receptors for ligand binding.

Six different type I serine/threonine kinase receptors have been identified in mammals (Ebner et al., Science 260, 1344-1348, 1993; Attisano et al., Cell 75, 671-680, 1993; Franzén et al., Cell 75, 681-692, 1993; ten Dijke et al., Oncogene 8, 2879-2887, 1993; ten Dijke et al., Science 264, 101-104, 1994; ten Dijke et

al., J. Biol. Chem. 269, 16985-16988, 1994; Tsuchida et al., Proc. Natl. Acad. Sci. U.S.A., 90, 11242-11246, 1993; Bassing et al., Science, 263, 87-89, 1994), which include a TGF-ß type I receptor (TßR-I), two activin type I receptors (ActR-I and ActR-IB) and two bone morphogenic proteins type I receptors (BMPR-IA and BPR-IB).

Bone morphogenic proteins (BMPs), also known as osteogenic proteins, are a family of proteins that induce ectopic bone formation at extraskeletal sites in vivo (reviewed in Reddi, Curr. Opinion Genet. Develop. 4, 737-744, 1994; Wozney, Prog. Growth Factor Res. 1, 267-280, 1989). BMPs act on osteoblasts and chondrocytes as well as other cell types, and they play important roles in embryonal development (reviewed in Harland, Proc. Natl. Acad. Sci. U.S.A. 91, 10243-10246, 1994). More than a dozen proteins belong to the BMP family, including BMP-2 to -6, osteogenic protein (OP)-1 (also termed BMP-7), OP-2, and growth/differentiation factor-5 to -7 (Kingsley, Genes and Dev. 8, 133-146, 1994; Massagué et al., Trends Cell Biol. 4, 172-178, 1994). BMP-4 binds to BMPR-IA and BMPR-IB efficiently (ten Dijke et al., J. Biol. Chem. 269, 16985-16988, 1994; Koenig et al., Mol. Cell. Biol. 14, 5961-5974, 1994) in the presence of DAF-4, a BMP type II receptor in Caenorhabditis elegans (Estevez et al., Nature 365:644-649, 1993), whereas OP-1 binds to BMPR-IB and, less efficiently, to BMPR-IA. Moreover, OP-1 can bind ActR-I in the presence of DAF-4 (ten Dijke et al., J. Biol. Chem. 269, 16985-16988, 1994).

Type II receptors for activin (ActR-II and ActR-IIB) and for TGF-ß (TßR-II) have been identified in mammals (Mathews and Vale, Cell, 65, 973-982, 1991; Mathews et al., Science 255, 1702-1705, 1992; Attisano et al., Cell, 68, 97-108, 1992; Lin et al., Cell 68, 775-785, 1992). The only BMP type II receptor identified to date is DAF-4 isolated from Caenorhabditis elegans (Estevez et al., Nature 365, 644-649, 1993).

Summary of the Invention

The present invention relates to the discovery of a novel mammalian TGF-β superfamily type II serine/threonine kinase receptor, referred to herein as U2 or BMPR-II, that binds bone morphogenic protein OP-1, but not activin. More

particularly, the invention is directed to an isolated, mammalian polypeptid capable of binding a bone morphogenic protein and having at least the extracellular amino acid sequence of the U2 receptor as shown in SEQ.ID.NO.1 or a functional derivative thereof. The novel U2 receptor has a serine/threonine kinase domain followed by a long serine and threonine-rich C-terminus. The long serine/threonine-rich region in the C-terminal end spans residues 513 to 1038 of SEQ.ID.NO.1.

The invention further relates to isolated nucleic acid molecules encoding the novel polypeptides. In one embodiment, the serine/threonine-rich C-terminus is encoded by nucleotides 1918 to 3495 of the nucleotide sequence encoding the novel U2 receptor as shown in SEQ.ID.NO.2. Complementary nucleic acids are also provided that can hybridize under stringent conditions to the isolated nucleic acid molecules encoding the novel polypeptides.

Vectors containing the nucleic acids of the present invention and host cells containing such vectors are also provided. Such vectors can also contain operational elements to express the polypeptides of the present invention.

The invention further provides methods for the recombinant production of the novel polypeptides by using DNA encoding polypeptides having binding affinity for a bone morphogenic protein such as OP-1.

Complexes containing the novel type II receptor of the present invention and a type I receptor of the TGF-β superfamily are also provided, where the type I receptor is ActR-1 or BMPR-1B. The complexes in the presence of 30-100 ng/ml of a bone morphogenic protein, such as OP-1, can transduce an intracellular signal.

Detailed Description of the Invention

The present invention relates to a novel type II receptor (U2 or BMPR-II) having greater binding affinity for bone morphogenic proteins, particularly OP-1, than activin. This novel receptor is a member of the TGF-β superfamily of receptors based on characteristics shared with known members of the TGF-β superfamily of receptors. For example, the U2 receptor has a hydrophobic signal peptide, an extracellular domain with a cyst ine pattern typical of other serine/threonine kinase receptors of the TGF-β superfamily receptor members, a hydrophobic transmembrane domain of 22 residues, and an intracellular serine/threonine kinase

domain of approximately 300 amino acids (residues 205 to 508 of SEQ.ID.NO.1).

The n vel U2 receptor, however, is distinguishable from other known TGF-β superfamily type II receptors. For example, the amino acid sequence of the human U2 receptor differs from other known receptors of the TGF-β superfamily. The human receptor also contains an extraordinarily long serine and threonine rich region in the C-terminal end that spans about 500 amino acids (residues 513 to 1038 of SEQ.ID.NO.1) in length compared with other known mammalian TGF-β superfamily type II receptors that contain serine and threonine rich regions in the C-terminus of less than about 115 amino acids. In addition, the U2 receptor has substantially greater binding affinity for OP-1, a bone morphogenic protein, compared with activin.

The present invention is accordingly directed to isolated, mammalian polypeptides having binding activity with a bone morphogenic protein and contains the amino acid sequence of the full length U2 receptor or a functional derivative thereof. As used herein, the term "polypeptide" is used synonymously with "protein." Likewise, the term "U2" is used synonymously with "BMPR-II."

An *E. coli* strain containing the nucleic acid molecule encoding the U2 receptor was deposited on May 20, 1994 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD (ATCC #69623) under the Budapest Treaty. This full length U2 receptor has the following amino acid sequence using the one-letter coding symbols:

(M)TSSLQRPWR VPWLPWTILL VSTAAASQNQ ERLCAFKDPY QQDLGIGESR
ISHENGTILC SKGSTCYGLW EKSKGDINLV KQGCWSHIGD PQECHYEECV
VTTTPPSIQN GTYRFCCCST DLCNVNFTEN FPPPDTTPLS PPHSFNRDET
IIIALASVSV LAVLIVALCF GYRMLTGDRK QGLHSMNMME AAASEPSLDL
DNLKLLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNF INEKNIYRVP
LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS
SCRLAHSVTR GLAYLHTELP RGDHYKPAIS HRDLNSRNVL VKNDGTCVIS
DFGLSMRLTG NRLVRPGEED NAAISEVGTI RYMAPEVLEG AVNLRDCESA
LKQVDMYALG LIYWEIFMRC TDLFPGESVP EYQMAFQTEV GNHPTFEDMQ
VLVSREKQRP KFPEAWKENS LAVRSLKETI EDCWDQDAEA RLTAQCAEER
MAELMMIWER NKSVSPTVNP MSTAMQNERN LSHNRRVPKI GPYPDYSSSS
YIEDSIHHTD SIVKNISSEH SMSSTPLTIG EKNRNSINYE RQQAQARIPS
PETSVTSLST NTTTTNTTGL TPSTGMTTIS EMPYPDETNL HTTNVAQSIG
PTPVCLQLTE EDLETNKLDP KEVDKNLKES SDENLMEHSL KQFSGPDPLS

STSSSLLYPL IKLAVEATGQ QDFTQTANGQ ACLIPDVLPT QIYPLPKQQN
LPKRPTSLAL NTKNSTKEPR LKFGSKHKSN LKQVETGVAK MNTINAAEPH
VVTVTMNGVA GRNHSVNSHA ATTQYANRTV LSGQTTNIVT HRAQEMLQNQ
FIGEDTRLNI NSSPDEHEPL LRREQQAGHD EGVLDRLVDR RERPLEGGRT
NSNNNNSNPC SEQDVLAQGV PSTAADPGPS KPRRAQRPNS LDLSATNVLD
GSSIQIGEST QDGKSGSGEK IKKRVKTPYS LKRWRPSTWV ISTESLDCEV
NNNGSNRAVH SKSSTAVYLA EGGTATTMVS KDIGMNCL. (SEQ.ID.NO.1)

The corresponding rat U2 receptor has the following amino acid sequence:

RYMAPQVLEG AVNLRDCESA LKQVDMYALG LIYWEVFMRC TDLFPGESVP
DYQMAFQTEV GNHPTFEDMQ VLVSREKQRP KFPEAWKENL AVRSLKETIE
ECWDHDPR. (SEQ.ID.NO.2)

As noted above, functional derivatives of the U2 receptor are also provided. As used herein, the term "functional derivative" means any biologically active modified form of the polypeptides. Such modifications can be (1) substitutions or additions in the amino acid sequence, and/or (2) the addition of a functional group to be used as a cross-linking agent or to improve certain pharmacokinetic or immunologic properties. For example, a functional derivative of the human U2 receptor can be the extracellular domain (residues 1 to 150 of SEQ.ID.NO.1) or biologically active fragments thereof that contains the active site for binding with OP-1. In addition, a functional derivative was prepared in which the serine/threonine kirase domain and the following serine/threonine-rich C-terminal region were deleted. This functional derivative was found to be biologically active in that it bound to OP-1. Such modifications, however, should not substantially decrease the binding affinity of the unmodified polypeptide for OP-1. Therefore, as used herein, the term "functional derivative" can mean an active fragment, an analog or a derivative that substantially retains the biological activity of the unmodified polypeptide. In the case of analogs, such modified polypeptides preferable have an amino acid homology of greater than about 40% compared to the U2 amino acid sequence or its extracelluar domain, more preferably in excess of 50%, and most preferably in excess of 90%. A molecule having an amino acid homology of about 99% is particularly useful.

One skilled in the art can readily make such modifications and test the binding activity of the modified form for OP-1 according to methods known in the art or as described in the Examples below. The modifications can be accomplished

by well known methods such as, for example, mutagenic techniques in which nucleotides are substituted or added that encode for a desired modification in the amino acid sequence. A general method is described, for example, in U.S. Patent No. 4,518,584, incorporated herein by reference.

The present invention further relates to nucleic acid molecules encoding the novel receptor or functional equivalents thereof. One nucleic acid molecule which encodes the novel human U2 receptor has the nucleotide sequence of SEQ.ID.NO.3, i.e.:

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GGCCTCCGCA CCCTGGATAT GTTTTCTCCC AGACCTGGAT ATTTTTTTGA
TATCGTGAAA CTACGAGGGA AATAATTTGG GGGATTTCTT CTTGGCTCCC  100
TGCTTTCCCC ACAGACATAC CTTCCGTTTG GAGGGCCGCG GCACCCCGTC
CGAGGCGAAG GAACCCCCCC ASCCGCGAGG GAGAGAAATG AAGGGAATTT  200
CTGCAGCGGC ATGAAAGCTC TGCAGCTAGG TCCTCTCATC AGCCATTTGT
CCTTTCAAAC TGTATTGTGA TACGGGCAGG ATCAGTCCAC GGGAGAGAAG  300
ACGAGCCTCC CGGCTGTTTC TCCGCCGGTC TACTTCCCAT ATTTCTTTTC
TTTGCCCTCC TGATTCTTGG CTGGCCCAGG G ATG ACT TCC TCG CTG  396
CAG CGG CCC TGG CGG GTG CCC TGG CTA CCA TGG ACC ATC CTG
CTG GTC AGC ACT GCG GCT GCT TCG CAG AAT CAA GAA CGG CTA 480
TGT GCG TTT AAA GAT CCG TAT CAG CAA GAC CTT GGG ATA GGT
GAG AGT AGA ATC TCT CAT GAA AAT GGG ACA ATA TTA TGC TCG 564
AAA GGT AGC ACC TGC TAT GGC CTT TGG GAG AAA TCA AAA GGG
GAC ATA AAT CTT GTA AAA CAA GGA TGT TGG TCT CAC ATT GGA 648
GAT CCC CAA GAG TGT CAC TAT GAA GAA TGT GTA GTA ACT ACC
ACT CCT CCC TCA ATT CAG AAT GGA ACA TAC CGT TTC TGC TGT 732
TGT AGC ACA GAT TTA TGT AAT GTC AAC TTT ACT GAG AAT TTT
CCA CCT CCT GAC ACA ACA CCA CTC AGT CCA CCT CAT TCA TTT 816
AAC CGA GAT GAG ACA ATA ATC ATT GCT TTG GCA TCA GTC TCT
GTA TTA GCT GTG TTG ATA GTT GCC TTA TGC TTT GGA TAC AGA 900
ATG TTG ACA GGA GAC CGT AAA CAA GGT CTT CAC AGT ATG AAC
ATG ATG GAG GCA GCA GCA TCC GAA CCC TCT CTT GAT CTA GAT 984
AAT CTG AAA CTG TTG GAG CTG ATT GGC CGA GGT CGA TAT GGA
GCA GTA TAT AAA GGC TCC TTG GAT GAG CGT CCA GTT GCT GTA 1068
AAA GTG TTT TCC TTT GCA AAC CGT CAG AAT TTT ATC AAC GAA
AAG AAC ATT TAC AGA GTG CCT TTG ATG GAA CAT GAC AAC ATT 1152
GCC CGC TTT ATA GTT GGA GAT GAG AGA GTC ACT GCA GAT GGA
CGC ATG GAA TAT TTG CTT GTG ATG GAG TAC TAT CCC AAT GGA 1236
TCT TTA TGC AAG TAT TTA AGT CTC CAC ACA AGT GAC TGG GTA
AGC TCT TGC CGT CTT GCT CAT TCT GTT ACT AGA GGA CTG GCT 1320
TAT CTT CAC ACA GAA TTA CCA CGA GGA GAT CAT TAT AAA CCT
GCA ATT TCC CAT CGA GAT TTA AAC AGC AGA AAT GTC CTA GTG 1404
AAA AAT GAT GGA ACC TGT GTT ATT AGT GAC TTT GGA CTG TCC
ATG AGG CTG ACT GGA AAT AGA CTG GTG CGC CCA GGG GAG GAA 1488
GAT AAT GCA GCC ATA AGC GAG GTT GGC ACT ATC AGA TAT ATG
GCA CCA GAA GTG CTA GAA GGA GCT GTG AAC TTG AGG GAC TGT 1572
GAA TCA GCT TTG AAA CAA GTA GAC ATG TAT GCT CTT GGA CTA
ATC TAT TGG GAG ATA TTT ATG AGA TGT ACA GAC CTC TTC CCA 1656
GGG GAA TCC GTA CCA GAG TAC CAG ATG GCT TTT CAG ACA GAG
GTT GGA AAC CAT CCC ACT TTT GAG GAT ATG CAG GTT CTC GTG 1740
TCT AGG GAA AAA CAG AGA CCC AAG TTC CCA GAA GCC TGG AAA
GAA AAT AGC CTG GCA GTG AGG TCA CTC AAG GAG ACA ATC GAA 1824
GAC TGT TGG GAC CAG GAT GCA GAG GCT CGG CTT ACT GCA CAG
TGT GCT GAG GAA AGG ATG GCT GAA CTT ATG ATG ATT TGG GAA 1908
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AGA AAC AAA TCT GTG AGC CCA ACA GTC AAT CCA ATG TCT ACT
GCT ATG CAG AAT GAA CGC AAC CTG TCA CAT AAT AGG CGT GTG 1992
CCA AAA ATT GGT CCT TAT CCA GAT TAT TCT TCC TCC TCA TAC
ATT GAA GAC TCT ATC CAT CAT ACT GAC AGC ATC GTG AAG AAT 2076
ATT TCC TCT GAG CAT TCT ATG TCC AGC ACA CCT TTG ACT ATA
GGG GAA AAA AAC CGA AAT TCA ATT AAC TAT GAA CGA CAG CAA 2160
GCA CAA GCT CGA ATC CCC AGC CCT GAA ACA AGT GTC ACC AGC
CTC TCC ACC AAC ACA ACA ACC ACA AAC ACC ACA GGA CTC ACG 2244
CCA AGT ACT GGC ATG ACT ACT ATA TCT GAG ATG CCA TAC CCA
GAT GAA ACA AAT CTG CAT ACC ACA AAT GTT GCA CAG TCA ATT 2328
GGG CCA ACC CCT GTC TGC TTA CAG CTG ACA GAA GAA GAC TTG
GAA ACC AAC AAG CTA GAC CCA AAA GAA GTT GAT AAG AAC CTC 2412
AAG GAA AGC TCT GAT GAG AAT CTC ATG GAG CAC TCT CTT AAA
CAG TTC AGT GGC CCA GAC CCA CTG AGC AGT ACT AGT TCT AGC 2496
TTG CTT TAC CCA CTC ATA AAA CTT GCA GTA GAA GCA ACT GGA
CAG CAG GAC TTC ACA CAG ACT GCA AAT GGC CAA GCA TGT TTG 2580
ATT CCT GAT GTT CTG CCT ACT CAG ATC TAT CCT CTC CCC AAG
CAG CAG AAC CTT CCC AAG AGA CCT ACT AGT TTG GCT TTG AAC 2664
ACC AAA AAT TCA ACA AAA GAG CCC CGG CTA AAA TTT GGC AGC
AAG CAC AAA TCA AAC TTG AAA CAA GTC GAA ACT GGA GTT GCC 2748
AAG ATG AAT ACA ATC AAT GCA GCA GAA CCT CAT GTG GTG ACA
GTC ACC ATG AAT GGT GTG GCA GGT AGA AAC CAC AGT GTT AAC 2832
TCC CAT GCT GCC ACA ACC CAA TAT GCC AAT AGG ACA GTA CTA
TCT GGC CAA ACA ACC AAC ATA GTG ACA CAT AGG GCC CAA GAA 2916
ATG TTG CAG AAT CAG TTT ATT GGT GAG GAC ACC CGG CTG AAT
ATT AAT TCC AGT CCT GAT GAG CAT GAG CCT TTA CTG AGA CGA 3000
GAG CAA CAA GCT GGC CAT GAT GAA GGT GTT CTG GAT CGT CTT
GTG GAC AGG AGG GAA CGG CCA CTA GAA GGT GGC CGA ACT AAT 3084
TCC AAT AAC AAC AAC AGC AAT CCA TGT TCA GAA CAA GAT GTT
CTT GCA CAG GGT GTT CCA AGC ACA GCA GCA GAT CCT GGG CCA 3168
TCA AAG CCC AGA AGA GCA CAG AGG CCT AAT TCT CTG GAT CTT
TCA GCC ACA AAT GTC CTG GAT GGC AGC AGT ATA CAG ATA GGT 3253
GAG TCA ACA CAA GAT GGC AAA TCA GGA TCA GGT GAA AAG ATC
AAG AAA CGT GTG AAA ACT CCC TAT TCT CTT AAG CGG TGG CGC 3336
CCC TCC ACC TGG GTC ATC TCC ACT GAA TCG CTG GAC TGT GAA
GTC AAC AAT AAT GGC AGT AAC AGG GCA GTT CAT TCC AAA TCC 3420
AGC ACT GCT GTT TAC CTT GCA GAA GGA GGC ACT GCT ACA ACC
ATG GTG TCT AAA GAT ATA GGA ATG AAC TGT CTG TGA 3498
AATGTTTTCA AGCCTATGGA GTGAAATTAT TTTTTGCATC ATTTAAACAT
GCAGAAGATG TTTACCGGGC GGGGTGACAG GAGAGAGCGT CAGCGGCAAG 3598
CTGTGGAGGA TGGGGCTCAG AATGCAGACC TGGGCTGGCC GCATGGCCTC
TCCCTGAGCC CTGATTTGTG GTAGGGAAGC AGTATGGGTG CAGTCCCCTC 3698
CTAGGCCTCC CTCTGGGGTC CCCCGGTCCT ATCCCACCTC TTCAGGGTGA
GCCAGCCTCA CCTCTSCCTA GTCCTGAGGG TGAGGGCAGG CTGAGGCAAC 3798
GAGTGGGAGG TTCAAACAAG AGTGGGCTGG AGCCAAGGGA AAATAGAGAT
GATGTAATTT CTTTCCGGAA TTC 3871
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The nucleic acid sequence encoding the rat U2 receptor has the sequence of SEQ.ID.NO.4, i.e.:

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CGT TAC ATG GCC CCT CAG GTG CTA GAA GGA GCT GTG AAC CTG
AGG GAC TGT GAG TCA GCA CTG AAG CAA GTG GAC ATG TAT GCG 84
CTT GGA CTG ATC TAC TGG GAG GTG TTT ATG AGG TGC ACA GAC
CTC TTC CCA GGT GAA TCT GTA CCA GAT TAC CAG ATG GCT TTT 168
CAG ACA GAA GTT GGA AAC CAT CCC ACA TTT GAG GAT ATG CAG
GTT CTT GTG TCA AGA GAA AAG CAG AGA CCC AAG TTC CCA GAA 252
GCC TGG AAA GAA AAT AGC CTG GCA GTG AGG TCA CTC AAA GAA
ACA ATC GAA GAG TGC TGG GAC CAC GAC CCC CGA 327
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As used h rein, the term "functional equivalent" means a modified nucleotide sequence having one or more additions, deletions, or substitutions to the above sequence that do not substantially affect the ability of the sequence to encode a polypeptide having OP-1 binding activity. Such modified sequences can be produced by means known in the art, including, for example, site directed mutagenesis.

The sequences can be obtained from natural sources, such as the natural DNA sequences encoding the extracellular domain of the U2 receptor protein. Alternatively, the sequences can be produced synthetically according to methods known in the art. Additionally, such DNA sequences can be derived from a combination of synthetic and natural sources. The natural sequences further include cDNA and genomic DNA segments. Methods of obtaining the synthetic and natural DNA sequences are described in PCT Publication No. WO 93/00431, published on January 7, 1993, which is incorporated herein by reference.

Nucleic acids that are complementary to the nucleic acids encoding the U2 receptor or its functional derivatives are also contemplated. Such complementary nucleic acids can be used, for example, as probes to hybridize under stringent conditions and, therefore, detect the U2 nucleic acids according to methods known to those skilled in the art. The term "stringent conditions" as used herein ref rs to parameters with which the art is familiar. More specifically, stringent conditions, as used herein, refers to hybridization in 1M NaCl, 1% SDS, and 10% dextran sulfate. This hybridization is followed by two washes of the filter at room temperature for 5 minutes in 2xSSC, and one wash for 30 minutes in 2xSSC, 0.1% SDS. There are other conditions, reagents and so forth that can be used, which result in the same or higher degree of stringency. One skilled in the art will be familar with such conditions.

The present invention further relates to methods of recombinantly producing the U2 receptor and its functional derivatives. The methods are accomplished by obtaining a DNA sequence encoding the desired U2 polypeptide, inserting the DNA sequence into a v ctor having operational elements for expression of the DNA, transferring the vector into a host cell capable of expressing the polypeptide,

culturing the host cell under conditions suitable for expression of the polypeptide, and harvesting the polypeptide.

The desired nucleic acid sequence can be inserted into a variety of vectors known to those skilled in the art using conventional methods. The nucleic acid sequence can be inserted and linked into a vector with any desired operational elements to effect its expression. The vectors can contain one or more of the following operational elements: (1) a promoter; (2) a Shine-Dalgarno sequence and initiator codon; (3) a terminator codon; (4) an operator; (5) sequence encoding a leader sequence to facilitate transportation out of the host cell; (6) a gene for a regulator protein; (7) a Kozak sequence preceding the initiator codon; and (8) any other DNA sequences necessary or preferred for appropriate transcription and subsequent translation of the vectors. EP Application No. 90 113 673.9, which is incorporated herein by reference, discloses several useful vectors and desirable operational elements.

The vectors can be transferred into suitable host cells by various methods known in the art, including transfection and transformation procedures. Various transfer methods are described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N.Y. (1989), which is incorporated herein by reference. Such host cells can be either eucaryotic or procaryotic cells. Examples of such host cells include chinese hamster ovary (CHO) cells, monkey kidney COS-1 cells, yeast, *E. Coli* and baculovirus infected insect cells. The host cells described in EP Application No. 90 113 673.9, which is incorporated herein by reference, are also useful in the present methods.

The host cells of the present invention can be cultured under conditions appropriate for the expression of the U2 polypeptide. These conditions are generally specific for the host cell and are readily determined by one of ordinary skill in the art in light of the published literature regarding the growth conditions for such cells. For example, Bergey's Manual of Determinative Bacteriology, 8th ed., Williams & Wilkins Co., Baltimore, Maryland, which is incorporated herein by reference, contains information relating to appropriate conditions for culturing bacteria. Similar information relating to culturing yeast and mammalian cells are

described in R. Pollack, Mammalian Cell Culture, Cold Spring Harbor Laboratories (1975), incorporated herein by reference.

The invention is further directed to complexes comprising the U2 type II receptor and a TGF-β superfamily type I receptor, such as ActR-1 or BMPR-IB. It has been found that the binding affinity for OP-1 in the complex is substantially increased compared to the OP-1 binding affinity of the U2 receptor alone or th type I receptors alone. The complex can further contain a bone morphogenic protein, such as OP-1.

The polypeptides of the present invention have a number of *in vitro* and *in vivo* uses. For example, the polypeptides can be used to affinity purify OP-1 from a number of sources, including serum from patients, cell culture supernatants or recombinantly produced OP-1, according to well known affinity purification procedures. Briefly, methods of purifying OP-1 from a sample are accomplished by: (a) contacting polypeptides of the present invention with the sample; (b) allowing the polypeptides to bind to OP-1; (c) dissociating the OP-1 from the polypeptides; and (d) collecting the dissociated OP-1. Conventional affinity purification methods can be used in which the polypeptides of the present invention are attached to resin, bead or other conventional matrix and placed in a column or other receptacle. The sample is then loaded and eluted with an appropriate solution that can readily be determined by those skilled in the art to obtain purified OP-1.

The polypeptides of the present invention can also be used as diagnostic reagents to detect or quantify OP-1 according to diagnostic methods well known in the art. Generally, methods of detecting or quantifying OP-1 in a sample is accomplished by (a) contacting polypeptides of the present invention with the sample suspected of containing OP-1; (b) allowing the polypeptides to bind to OP-1; and (c) detecting or quantifying OP-1 bound to the polypeptides.

The polypeptides of the present invention can further be used in combination with type I receptors, particularly ActR-I or BMPR-1B, to detect or quntify OP-1 in samples, such as biological fluids for example. Generally, methods of detecting or quntifying OP-1 in a sample can be accomplished by (a) co-transfecting a mammalian cell line, such as monkey kidney COS-1 cells or chinese hamster ovary cells (CHO), with plasmids capable of expressing U2 and ActR-I or BMPR-1B, and

a suitable reporter plasmid such as p3TP-Lux capable of expressing a quantifiable enzyme activity, such as luciferase; (b) contacting the transfected cells with a sample suspected of containing Op-1; (c) allowing the transfected cells to bind OP-1; and (d) detecting or quantitating the amount of enzyme activity produced in the cultures. Commercially available detection kits can be used in these methods.

The polypeptides can also be used as immunogens to produce polyclonal and monoclonal antibodies according to procedures well known in the art and as described, for example, in Harlow & Lane, Antibodies: A Laboratory Manual (1988), incorporated herein by reference. Such antibodies can, in turn, be used to detect the U2 receptors or receptor complexes for *in vitro* as described above and *in vivo* uses such as imaging according to procedures known in the art.

The following examples are intended to illustrate, but not limit, the present invention.

EXAMPLE 1

Cloning of a cDNA for BMPR-II (U2)

Comparison of peptide sequences of the human transforming growth factor - beta (TGF-β) type II receptor (Lin et al., Cell 68:775-785, 1992), murine activin type II receptor (Mathews and Vale, Cell 65:973-982, 1991), and the murine TGF-β type I receptor (Ebner et al.) revealed conserved peptide regions within the kinase domain. Conserved regions with specificity to membrane bound serine /threonine kinases were selected for the design of degenerate oligonucleotide primers. EcoRI restriction sites were added to the 5' ends of the primers for subsequent cloning purposes and the oligonucleotides were synthesized of an Applied Biosystems 391 DNA synthesizer (Applied Biosystems, Inc., Foster City, CA). Amplification of cDNA via polymerase chain reaction (PCR) employing the following oligonucleotide primers provided a means of selective amplification of related sequences:

Plasmid	Oligonucleotide Sequence
TR-A	5'GGGAGGGAATTC GTNGCNGTNAARATHTTYCC3' (SEQ.ID.NO.5)
TR-B	5'GGGAGGGAATTC CAYGARAAYATHYTNCARTT3' (SEQ.ID.NO.6)

Plasmid	Oligonucleotide Sequence
TR-C	5'GGGAGGGAATTC TGGYTNATHACIGCNTWYCAYG3' (SEQ.ID.NO.7)
TR-CR	5'GGGAGGGAATTC CRTGRWANGCiGTDATNARCCA3' (SEQ.ID.NO.8)
TR-D	5'GGGAGGGAATTC MGNTAYATGGCNCCNCARGTiYT3' (SEQ.ID.NO.9)
TR-DR	5'GGGAGGGAATTC ARNACYTGNGGNGCCATRTAiC3' (SEQ.ID.NO.10)
TR-ER	5'GGGAGGGAATTC TCNGSRTYITGRTMCCARCAYTC3' (SEQ.ID.NO.11)

In the above oligonucleotide primer sequences, H = C,A or T; W = A or T; D = A,G or T; M = A or C; S = G or C; N = A,C,G, or T; R = A or G; Y = C or T; and i = Inosine.

EXAMPLE 2

Identification of a Novel Serine/Threonine Kinase Receptor

Poly A$^+$ RNA was isolated from 25 mg of adult rat substantia nigra tissue using a Quick Prep mRNA Purification Kit (Pharmacia, Ippsala, Sweden). Single strand cDNA was synthesized as follows; 1μg polyA$^+$ RNA and 0.5μg oligo d(T) (BRL, Gaithesburg, MD) in 10μl H_2O were heated to 70°C for 10min, then put on ice. The cDNA synthesis reaction was performed in 50mM Tris-HCl pH 8.3, 75mM KCl, 3mM $MgCl_2$, 10mM dithiothreitol, 0.2mM each of dATP, dCTP, dGTP, and dTTP, 1 unit Human Placental Ribonuclease Inhibitor (BRL), and 200 units Moloney Murine Leukemia Virus reverse transciptase (BRL) in 20μl total volume at 42° C for one hour. The reaction was then diluted to 40μl with H_2O and stored at -70° C. PCR was performed in 10mM Tris pH 8.3, 50 mM KCl, 0.001%, bovine serum albumin (BSA), 1.5mM or 2.5mM $MgCl_2$, 50pmol of the TR-D and TR-ER prim rs, 0.2mM each of dATP, dCTP, dGTP, and dTTP, 2.5 units AmpliTaq DNA Polymerase (Roche Molecular Systems, Branchburg, N.J.), and 3μl of single strand cDNA template at a volume of 40μl which was then used in the PCR Gem hot start method as set forth herein. The reaction mixtures were heated to 95°C for 5

minutes and 35 amplification cycles were carried out (95°C 1 min, 45°C 1 min, 72°C 1 min), followed by a final 10 minute extention reaction at 72°C. The product band of the TR-D and TR-ER primed PCR reaction was in the predicted size range of 300 - 350 bp. This band was size selected on a 1% agarose, 1.5% Nuseive (FMC, Rockland, ME) gel in TAE buffer. The product band was excised and DNA was extracted with a Qiaex Gel Purification Kit (Qiagen, Chatsworth, CA) following the manufacturer's instructions. The DNA fragment was then digested with EcoRI, and ligated to EcoRI-digested and phosphatase treated pBluescriptII SK plasmid DNA (Stratagene, San Diego, CA). A sample of the ligation product was used to transform *E. coli* strain XL1Blue (Stratagene) by electroporation. Transformed colonies were selected by plating the bacteria with 10μl 100mM isopropylthio-β-galactoside, and 100μl 2% 5-bromo-4-chloro-β-D-galactoside on LB pH 7 agar plates containing 50 μg/ml ampicillin. Recombinant colonies were analyzed for inserts by PCR using T3$^+$, T7$^+$, SK$^+$, or KS$^+$ primers flanking the polylinker. The following oligonucleotide primers were synthesized on an ABI 360 DNA synthesizer (ABI) for use in PCR and sequencing:

SK$^+$ 5'CGCTCTAGAACTAGTGGATCC3' (SEQ.ID.NO.12)

KS$^+$ 5'TCGAGGTCGACGGTATCGATA3' (SEQ.ID.NO.13)

T3$^+$ 5'ATTAACCCTCACTAAAGG3' (SEQ.ID.NO.14)

T7$^+$ 5'TAATACGACTCACTATAGG3' (SEQ.ID.NO.15)

Individual bacterial colonies were toothpicked into 10μl H_2O for 1min., then the toothpick was streaked on an LB agar plates containing 50μg/ml ampicillin to make a plate stocks. A PCR reaction mix was added to the resuspended bacteria to a final concentration of 10mM Tris pH 8.3, 50mM KCl, 0.001% BSA, 2.0mM $MgCl_2$, 25pmol each of the flanking 5' and 3' primers, and 1.25 units AmpliTaq DNA Polymerase in a total reaction volume of 40μl. Reactions were heated at 95 C for 3min and then received 25 cycles of (95 C 1min, 48 C 1min, and 72 C 1min) and a 10min extension at 72°C. Samples of the PCR reaction were electrophoresed on a 1% agarose (BRL)/ 1.5% Nuseive agaraose (FMC) gel. These reactions with the expected size inserts, 300 to 350bp, were purified with a Wizard DNA Clean-up Kit (Promega, Madison, WI) to remove remaining nucleotides, primers and enzyme.

A Dye-Deoxy Terminator Sequencing Kit (ABI) and T3$^+$, T7$^+$, SK$^+$, or KS$^+$ primers outside the insert were used to determine the DNA sequence of the PCR products.

Analysis of the resulting sequences revealed multiple copies of one sequence that encoded a novel, putative serine/threonine kinase. This isolate was designated rat U2 and the DNA sequence and deduced amino acid sequence are shown in SEQ.ID.NO.3 and SEQ.ID.NO.4. In addition four other previously identified serine/threonine kinase receptors, as well as TGF-β type II and activin type II receptors, were found.

The use of degenerate oligonucleotide primers, TR-A with TR-CR or TR-DR, and TR-B with TR-DR, from other conserved regions of the kinase domain in this protocol gave no product band of the predicted size. When the TR-C and TR-ER primer pair was used the only clones obtained were TGF-β type II and activin type II receptors.

EXAMPLE 3

Identification and sequence of a human U2 cDNA

To find a full length cDNA encoding the human U2 receptor, a human substantia nigra cDNA library in λgt10 (Clontech, Palo Alto, CA) was hybridized with a ^{32}P-labeled rat U2 probe. The PCR fragment from one rat U2 containing plasmid was agarose gel purified with a Qiaex Gel Extraction Kit (Qiagen). A PCR protocol was used to ^{32}P-label the U2 fragment. Three ng of rat U2 fragment were used in a reaction with 60pmol SK$^+$ and KS$^+$ primers; 0.2mM dATP, dGTP, and dTTP; 0.0125mM dCTP; 50pmol α^{32}P-dCTP ~3000Ci/mmol; 5 units AmlpiTaq DNA polymerase (Perkin Elmer Cetus); containing 10mM Tris pH 8.3, 50mM KCl, 2.0 mM $MgCl_2$, 0.001% BSA; in 50μl total reaction volume. The reaction was heated to 95 C for 5 min then 25 cycles at (95°C 1 min, 62°C 1 min, and 72°C 1 min) were carried out. Unicorporated nucleotides were removed on a Bio Spin 30 column (Bio-Rad, Richmond, CA) . The human substantia nigra cDNA library was plated, using the *E. coli* strain C600, on LB agar plates with NZCYM top agarose at a density of 30,000 plaques per 150mm plate. A total of 720,000 plaques were plated for screening. Duplicate nitrocellulose filter lifts of the plaques were made using standard protocols (Maniatis, et al). The filters were hybridized in 40%

formamide, 0.90 M NaCl, 0.05M $NaPO_4$, 5mM EDTA (pH 7.4), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.1% SDS, 100μg/ml yeast tRNA, with the rat U2 probe at $1x10^6$ cpm/ml hybrididation solution at 42 C overnight. Filters were washed two times in 0.36M NaCl, 20mM sodium phosphate buffer, 2mM EDTA (pH7.4), and 0.1% SDS at room temperature and then two times in 36mM NaCl, 2mM sodium phosphate buffer, 0.2mM EDTA (pH 7.4), and 0.1% SDS at 60°C and exposed to photographic film. Positive plaques were picked, replated, and rehybridized as above to isolate individual positive plaques.

After the λgt10 clones were plaque purified. their insert sizes were determined by PCR amplification with λgt10-5' and λgt10-3' oligonucleotide primers (Clontech). The insert sizes of five of the cDNA clones ranged from 1.0 kb to 4.0 kb. The coding orientation within the λ phage and size of the insert upstream of U2A,an oligonucleotide internal to the rat U2 clone, was determined by PCR with the U2A primer U2A (5'TTGAG TGACC TCACT GCCAG GC) (SEQ.ID.NO.14) and either the λgt10-5' or λgt10-3' flanking primer. U2A gave a major PCR product band in combination with only one flanking primer and artifact bands with the other flanking primer. This allowed the orientations of the cDNAs in the phages to be determined. From the resulting product bands of these PCR's a set of overlapping cDNA clones was deduced. Partial sequencing of the U2 cDNA was accomplished by using the flanking primers with these PCR products in Dye-Deoxy Terminator Sequencing reactions (ABI). This facilitated the synthesis of specific internal oligonucleotides for use as primers in sequencing the full U2 cDNA.

The largest insert, 4kb, in clone λ14-1was chosen to be subcloned into pBluescriptII SK$^-$ (Stratagene) plasmid vector for complete sequencing. Phage particles from a 50 ml liquid lysate of λ14-1 were pelleted by high speed centrifugation, resuspended in 0.5ml of 0.3M NaCl, 20mM EDTA, 0.5% SDS, digested with 0.5μg/ml proteinase K at 55°C for one hour, and extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), then extracted with an equal volume of chloroform:isoamyl alcohol (24:1), and DNA isolated by ethanol precipitation. The insert in λ14-1 was excised by EcoRI digestion, electrophoresed on a 0.8% agarose gel in TAE buffer, and the 4kb cDNA insert isolated using a Qiaex Gel Extraction Kit (Qiagen). This 4kb EcoRI DNA fragment was ligated into

Ec RI digested, dephosphorylated pBluescriptII SK- plasmid DNA (Stratag ne). One transformant, pSK/U2-27, with the 4kb insert was identified by PCR methods as detailed above. Plasmid DNA of pSK/U2-27 was prepared using a Plasmid Mini Kit (Qiagen). The DNA sequence of the 4kb U2 cDNA was determined using a series of oligonucleotide primers in Dye-Deoxy Terminator Sequencing Kit (ABI) reactions. The full-length coding sequence of U2 and the translation for the polypeptide it encodes, along with 5' and 3' flanking DNA sequences are shown in SEQ.ID.NO.1. The human U2 polypeptide has typical features of TGF-β super-family member receptors; a hydrophobic signal peptide, residues 1 to 26, with a presumed cleavage site after residue 26; an extracellular domain, residues 27 to 150, with a cysteine pattern typical of other serine/threonine kinase receptors; three potential N-linked glycosylation sites (Asn-Xaa-Ser/Thr, where Xaa can be any amino acid); a hydrophobic transmembrane domain of 22 residues; and an intracellular serine/threonine kinase domain of approximately 300 residues.

As discussed above, the cDNA of U2 encodes a protein of 1038 amino acid residues containing an N-terminal hydrophobic leader sequence, followed by an extracellular domain, a single transmembrane domain, and an intracellular domain with a serine/threonine kinase region. The U2 protein lacks a glycine-and serin -rich sequence in the juxtamembrane domain, which is typical for type I receptors (Massagué et al., Trends Cell Biol., 4, 172-178, 1994; Kingsley, Genes and Dev. 8, 133-146, 1994), and is considerable larger (a molecular mass of about 115 kDa) than previously described serine/threonine kinase receptors due to the presence of a long C-terminal tail rich in serine and threonine residues (22% of all amino acids in this region). The long C-terminal tail rich in serine and threonine comprises residues 513 to 1038 of SEQ.ID.NO.1.

Comparison of the amino acid sequence of the kinase domain of U2 revealed that it is likely to have specificity for serine/threonine residues, but it is only distantly related to the other serine/threonine kinases, including DAF-4. There are ten cysteine residues in the extracellular domain, which could be well aligned with cysteine residues in the other serine/threonine kinase receptors; however, the amino acid sequence identity in the extracellular domain of U2 is less than 28% compared

to other serine/threonine kinase receptors. No sequence similarity between the long C-terminal tail and other known sequences was found.

EXAMPLE 4

Expression of U2 RNA

Poly A^+ RNA, 2μg, isolated from rat substantia nigra tissue as described above was electrophoresed on a 1.25% agarose gel in HEPES, 37% formaldehyde buffer and capillary- transferred to Hybond N membrane (Amersham) in 3.0M NaCl, 0.3M sodium citrate (pH 7.0). The RNA was bound to the filter by baking at 80°C for 2 hours. The 4 kb PCR fragment of human U2 in pSK/U2-27 was gel isolated and labeled with α^{32}P dCTP by random primer synthesis with a Quick Prime Kit (Pharmacia, Piscataway, N.J.). Hybridization of this probe to the rat substantia nigra Northern blot was performed in 40% formamide, 1.08 M NaCl, 0.06M $NaPO_4$, 6mM EDTA (pH 7.4), 0.04% Ficoll, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin, 0.1% SDS, 100μg/ml yeast tRNA with $2x10^6$ cpm at 42°C overnight. The membranes were then washed two times in 0.36M NaCl, 20mM $NaPO_4$, 2mM EDTA (pH7.4), 0.1% SDS at room temperature, and then two times in 36mM NaCl, 2mM $NaPO_4$, 0.2mM EDTA (pH 7.4), 0.1% SDS at 60°C and autoradiographic exposures made for 1 to 9 days. The most prominent transcript was 11kb, with minor transcripts of 10kb and 5.4kb.

Human Multiple Tissue Northern blots I and II (Clontech, Palo Alto, CA), and a Human Brain Multiple Tissue Northern blot (Clontech) were hybridized with the same U2 probe in 50% formamide, 0.90 M NaCl, 0.05M $NaPO_4$, 5mM EDTA (pH 7.4), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.1% SDS, 100μg/ml yeast tRNA; or in 50% formamide, 1.08M NaCl, 0.06M $NaPO_4$, 6mM EDTA (pH 7.4), 0.04% Ficoll, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin, 0.1% SDS, 100μg/ml yeast tRNA; with $2x10^6$ cpm/ml U2 probe at 42°C overnight. Filters were washed two times in 0.36M NaCl, 20mM $NaPO_4$, 2mM EDTA (pH7.4), 0.1% SDS at room temperature and the two times in 36mM NaCl, 2mM $NaPO_4$, 0.2mM EDTA (pH 7.4), 0.1% SDS at 68 C and autoradiographic exposures were made for two to nine days.

U2 mRNA is expressed at the high st levels in brain and skeletal muscle with all brain tissues tested; amygdala, caudate nucleus, corpus callosum, hippocampus, hypothalamus, substantia nigra, subthalamic nucleus, and thalamus; expressing approximately equal levels. U2 transcripts were also detected in heart, kidney, lung, placenta, testis, pancreas, ovary, prostate, and small intestine. The 11kb transcript was the most prominent band in each tissue; the 10kb and 5.4 transcripts were detected only in the highest expressing tissues.

Additional Northern blots were prepared using different hybridization conditions. Poly A+ RNAs from human adult caudate nucleus, hippocampus, substantia nigra, whole brain, kidney, lung and human fetal whole brain, fetal kidney and fetal lung (Clontech) were electrophoresed on agarose-formaldehyde gels and tansferred to Hybond N. membranes (Amersham, Arlington Heights, IL). A portion of the human U2 cDNA from 93 bp upstream of the ATG condon to bp 2518 was PCR amplified and ^{32}P-labeled using a Quick Prime Kit (Pharmacia). Hybridization conditions were 0.5 M. $NaPO_4$, pH 7.4, 7% SDS, 1 mM EDTA, 200 μg/ml yeast tRNA at 68°C overnight and washed as above with high temperature washes at 68°C. The results obtained from this blot gave better resolution of transcripts. Major transcripts of 11.5 kb, 7.7 kb, and 5.0 kb were present in all tissues and a m' or transcript of 6.6 kb was seen in some tissues.

EXAMPLE 5

U2 is a single copy human gene

A Southern blot of human genomic DNA (Clontech) restriction endonuclease disgested with BamHI was prepared. The blot was hybridized with the ^{32}P-labeled rat U2 PCR probe, and the same hybridization and wash conditions, described for the cDNA library screen. Autoradiographic exposures of the washed filter gave only one band greater than 20kb, indicating U2 is most likely a single copy gene.

EXAMPLE 6

Binding of OP-1 and U2

In order to identify ligands for U2, binding studies using ^{125}I-labeled members of the TGF-ß superfamily were performed. cDNAs for U2 and various type I

receptors were transfected singly or together into COS-1 cells; cells were then incubat d with various ^{125}I-labeled ligands, washed and subjected to cross-linking with a homobifunctional cross-linker. Samples were then analyzed by SDS-gel electrophoresis after immunoprecipitation using antisera to type II or type I receptors. The methods used are described below.

For transient transfection, cDNAs for type I receptors (ten Dijke et al., Oncogene 8, 2879-2887, 1993; ten Dijke et al., Science 264, 101-104, 1994) or type II receptors (DAF-4 obtained from D.L. Riddle, University of Missouri, Missouri, or U2) subcloned into pSV7d (Truett et al., DNA 4, 333-349, 1985), pcDNA3 (Invitrogen, San Diego, CA) or pCMV5 (Andersson et al., J. Biol. Chem. 264, 8222-8229, 1989) expression vectors, were used. These plasmids were transfected into COS-1 cells by a calcium phosphate precipitation method using an MBS mammalian transfection kit (Stratagene, La Jolla, CA), as previously described (ten Dijke et al., Science 264, 101-104, 1994: Okadome et al., J. Biol. Chem.(in press), 1994). The COS-1 cells (catalogue #CRL-1650) were obtained from American Type Culture Collection (Rockville, MD). The cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and antibiotics (100 units/ml penicillin and 50 μg/ml streptomycin) in a 5% CO_2 atmosphere at 37°C. The transfected cells were incubated on ice for 2-3 h with 0.2-0.5 nM of ^{125}I-labeled ligands in the presence or absence of unlabeled ligands in the binding buffer (phosphate-buffered saline containing 0.9 mM $CaCl_2$, 0.49 mM $MgCl_2$ and 1 mg/ml bovine serum albumin). After incubation, the cells were washed with the binding buffer without bovine serum albumin and cross-linking was done in the same buffer containing 1 mM bis(sulfosuccinimidyl) suberate (BS^3) (Pierce Chemical Co., Rockford, IL) for 15 min on ice. The cells were washed once with 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10% glycerol and 0.3 mM phenylmethylsulphonyl fluoride. The cells were scraped off the plates in the same buffer, centrifuged and resuspended in solubilization buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.3 mM phenylmethylsulphonyl fluoride, 1.5% Trasylol, 1% Triton X-100 and 1% deoxycholate), followed by incubation for 20 min on ice. Immunoprecipitation of the cross-linked materials was performed as described (Yamashita et al., J. Biol. Chem. 269, 20172-20178, 1994). The immune

complexes were separated by boiling 3 min in SDS-sample buffer with 10 mM dithiothreitol, and subjected to SDS-gel electrophoresis, followed by analysis using a PhosphorImager (Fuji film).

Antisera to type I receptors were made against synthetic peptides corresponding to the intracellular juxtamembrane parts of type I receptors as previously reported (ten Dijke et al., Science 264, 101-104, 1994). Antisera against U2 (referred to as SMN and NRR) were generated against peptides corresponding to amino acid residues 185-202 and 534-556, respectively. Peptid s were coupled to keyhole limpet hemocyanin and injected into rabbits as described (ten Dijke et al., Science 264, 101-104, 1994). SMN and NRR antisera recognized U2 equally well; therefore, a mixture of SMN and NRR (1:1) was used, unless specified.

Recombinant human OP-1 (Sampath et al., J. Biol. Chem. 267, 20352-20362, 1992), TGF-ß1 and activin A, were obtained from T. Kuber Sampath (Creative BioMolecules, Hopkington, MA), H. Ohashi (Kirin Brewery Company, Japan) and Y. Eto (Ajinomoto Company, Inc., Japan), respectively. Recombinant GDNF was obtained as described (Lin et al., Science 260, 1130-1132, 1993). OP-1 was iodinated using the chloramine T method according to Frolik et al.(J. Biol. Chem. 259, 10995-11000, 1984) but chloramine T was added two times instead of three times (ten Dijke et al., J. Biol. Chem. 269, 16985-16988, 1994). ^{125}I-OP-1 was observed by SDS-PAGE as multiple components of 16-19 kDa under reducing conditions.

Using the above procedures, U2 alone was found to bind ^{125}I-labeled OP-1 efficiently, but did not bind TGF-ß1, activin A, or GDNF. Affinity cross-linking studies using ^{125}I-OP-1 revealed that U2 bound OP-1 and formed a cross-linked complex of 130 kDa, and a less abundant complex of 140 kDa. When the cDNA for U2 was co-transfected with cDNAs for ActR-I or BMPR-IB, which are known to bind ^{125}I-OP-1 in the presence of DAF-4 (ten Dijke et al., J. Biol. Chem., 269, 16985-16988, 1994), the abundance of the 130 kDa complex was increased and complexes of 220, 190, 95 and 76-80 kDa, could also be seen. The fact that all complexes were immunoprecipitated by antisera against both the type I receptors and with antisera to type II receptor, indicates that ligand binding induces a

complex of type I and type II receptors. The 80-76 kDa components represent type I receptors (ActR-I or BMPR-IB) (ten Dijke et al., J. Biol. Chem., 269, 16985-16988, 1994). The other components may represent oligomer(s) of U2 and type I receptors, or receptors cross-linked to the OP-1 dimer. Similar multiple bands have also been identified when the COS-1 cells were transfected with DAF-4 cDNA together with the corresponding type I receptors (ten Dijke et al., J.Biol. Chem. 269, 16985-16988, 1994). The binding of ^{125}I-OP-1 was completed by excess amounts of unlabeled OP-1, but not by TGF-ß1, activin A or GDNF.

The binding of ^{125}I-OP-1 to U2 was compared with the binding to C. elegans BMP type II receptor DAF-4 in the presence of type I receptors. ^{125}I-OP-1 bound both of the type II receptors; cross-linked complexes of U2 migrated slightly slower than those of DAF-4 on SDS-PAGE, consistent with the smaller molecular mass of DAF-4 compared to U2.

EXAMPLE 7

Expression of U2 in Cultured Cell Lines

It has previously been shown that ^{125}I-labeled BMPs bind BMPR-IA, BMPR-IB or ActR-I in certain cultured cell lines, including mink lung epithelial (Mv1Lu) cells and U-1240MG glioblastoma cells (ten Dijke et al., J. Biol. Chem. 269, 16985-16988, 1994). The binding of ^{125}I-OP-1 to receptors endogenously expressed in these cultured cells were studied using antiserum to U2 (NRR), the preparation of which is described above. The U-1240MG glioblastoma cells (Nistér et al., Cancer Res. 48, 3910-3918, 1988) were obtained from B. Westermark (Uppsala University, Sweden). Mv1Lu cells (catalogue #CCL-64) were obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and antibiotics (100 units/ml penicillin and 50 ug/ml streptomycin) in a 5% CO_2 atmosphere at 37°C.

The cells were incubated with ^{125}I-OP-1 and treated with a cross-linking reagent as described above. Immunoprecipitation of the cross-linked complexes followed by SDS-gel electrophoresis revealed that type II receptor complexes of 130 kDa, as well as other components, which may represent co-immunoprecipitated

type I receptors and oligomer(s) of type I and/or type II receptors, could be observed in R mutant Mv1Lu cells, which are described in Example 8, and U-1240MG glioblastoma cells. Experiments with wild type Mv1Lu cells gave similar results as with R mutant cells. A component of 165 kDa also was seen in these cells; whether this component represents an oligomer of U2 and/or type I receptors or an alternatively spliced variant of U2, remains to be elucidated. Similar results were obtained using another U2 antiserum (SMN). Previous studies have shown that when transfected into COS-1 cells, BMPR-IB bound ^{125}I-I-OP-1 in the absence of a co-transfected type II receptor (ten Dijke et al., J. Biol. Chem. 269, 16985-16988, 1994). In order to test whether this may be due, at least in part, to the presence of endogenously expressed U2 in COS-1 cells, cDNA for BMPR-IB was transfected into COS-1 cells, the cells were incubated with ^{125}I-OP-1, cross-linked and cross-linked complexes were immunoprecipitated by antiserum to U2. A cross-linked complex of 130 kDa could be immunoprecipitated by the U2 antis rum in these cells, supporting the notion that COS-1 cells endogenously express U2. COS-1 cells were also found to endogenously express mRNA for U2 by Northern blot analysis using methods described in Example 4.

EXAMPLE 8

Signaling Activity of U2

The signaling activity of U2 was investigated using a p3TP-Lux promoter-reporter construct (Wrana et al., Cell 71, 1003-1014, 1992; Attisano et al., Cell 75, 671-680, 1993). R mutant Mv1Lu cells, which are highly transfectable and suitable for the p3TP-Lux assay, were used. The R mutant cell line (clone 4-2 ;Laiho et al., J. Biol. Chem. 265, 18518-18524, 1990) was created by chemical mutagenesis of the Mv1Lu cell line and was a gift from M. Laiho (University of Helsinki, Finland) and J. Massagué (Memorial Sloan-Kettering Cancer Center, New York, NY). Although the R mutant cells express endogenous receptors for activins (ten Dijke et al., Science 264, 101-104, 1994) and BMPs, superinduction of transcriptional signals could be detected after the co-transfection of cDNAs for ActR-II and ActR-I in this assay system (Attisano et al., Cell 75, 671-680, 1993; Yamashita et al., submitted for publication). The cells were cultured in Dulbecco's

modified Eagle's medium containing 10% fetal bovine serum and antibiotics (100 units/ml penicillin and 50 ug/ml streptomycin) in 5% CO_2 atmosphere at 37°C. The R mutant Mv1Lu cells were co-transfected with the p3TP-Lux promoter-reporter construct and plasmids containing type II or type I receptor cDNAs as described above. Cells were washed with phosphate-buffered saline on the following day. The cells were starved in Dulbecco's modified Eagle's medium containing 0.1% fetal bovine serum and antibiotics (100 units/ml penicillin and 50 μg/ml streptomycin) for 6 h and then exposed to various concentrations of OP-1 for 24 h. Luciferase activity in the cell lysate was measured using the luciferase assay system (Promega, Madison, WI) according to the manufacturer's protocol and a luminometer (model 1250; LKB Pharmacia, Piscataway,NJ).

When R mutant cells were transfected with a single receptor cDNA (U2, ActR-I or BMPR-IB) together with the p3TP-Lux plasmid, transcription , as measured by an increase in luciferase activity above control levels, was not activated aft r addition of OP-1 (Table I). When the p3TP-Lux plasmid was co-transfected with U2 and a type I receptor (ActR-I or BMPR-IB) into R mutant cells, transcriptional activation was observed after stimulation by OP-1 (Table I), as evidenced by an increase in luciferase activity above control levels. Transcriptional activation in cells transfected with cDNAs for DAF-4 and ActR-I or U2 alone was not significant. These results indicate that U2 binds OP-1 and transduces signals together with the corresponding appropriate type I receptors.

Table I

cDNA transfected	OP-1 (ng/ml)	Luciferase activity (% of control)
U2	0	100
	100	117
ActR-I	0	100
	100	129
BMPR-IB	0	100
	100	115
U2 + ActR-I	0	100
	30	212
	100	394
U2 + BMPR-IB	0	100
	30	160
	100	213
DAF-4 + BMPR-IB	0	100
	30	95
	100	124
DAF-4 + ActR-I	0	100
	30	81
	100	94

The foregoing description of the invention is exemplary for purposes of illustration and explanation. It will be apparent to those skilled in the art that changes and modifications are possible without departing from the spirit and scope of the invention. It is intended that the following claims be interpreted to embrace all such changes and modifications.

What is claimed is:

1. An isolated, mammalian polypeptide having binding affinity for a bone morphogenic protein (BMP) and having the amino acid sequence of SEQ.ID.NO.1, SEQ.ID.NO.2, or a functional derivative thereof.

2. The isolated polypeptide of claim 1, wherein said polypeptide is a human type II receptor of the TGF-β superfamily.

3. The isolated polypeptide of claim 1, wherein said functional derivative is the extracellular domain of said amino acid sequence or an active fragment thereof.

4. The isolated protein of claim 1, wherein said BMP is osteogenic protein-1 (OP-1).

5. An isolated nucleic acid molecule encoding the polypeptide of claim 1.

6. The isolated nucleic acid molecule of claim 5 having the nucleotide sequence of SEQ.ID.NO.3, SEQ.ID.NO.4 or a functional equivalent thereof.

7. The isolated nucleic acid molecule of claim 6, wherein said functional equivalent encodes the extracellular domain of said polypeptide or an active fragment thereof.

8. The isolated nucleic acid molecule of claim 5, wherein the nucleic acid encodes a human type II receptor of the TGF-β superfamily having binding activity with the BMP.

9. The isolated nucleic acid molecule of claim 8, wherein the bone morphogenic protein is osteogenic protein is OP-1.

10. The isolated nucleic acid molecule of claim 5, wherein said nucleic acid is DNA.

11. The isolated nucleic acid molecule of claim 10, wherein said nucleic acid molecule is cDNA.

12. The isolated nucleic acid molecule of claim 10, wherein said nucleic acid molecule is genomic DNA.

13. A complementary nucleic acid molecule which hybridizes to the isolated nucleic acid molecule of claim 5 under stringent conditions.

14. A vector containing the nucleic acid molecule of claim 5.

15. The vector of claim 14, wherein said nucleotide sequence further contains operational elements to express th polypeptide.

16. A recombinant host cell comprising the vector of claim 14.

17. A method for producing a polypeptide having binding activity with a bone morphogenic protein comprising:

(a) obtaining a nucleic acid molecule encoding the polypeptide of claim 1;

(b) inserting the DNA sequence into a vector having operational elements for expression of the DNA;

(c) transferring the vector into a host cell capable of expressing th polypeptide;

(d) culturing the host cell under conditions favorable for expression of the polypeptide; and

(e) harvesting the polypeptide.

18. A complex of the polypetide of claim 1 and a type I receptor of the TGF-β superfamily.

19. The complex of claim 18, wherein the type I receptor is ActR-1.

20. The complex of claim 18, wherein the type I receptor is BMPR-1B.

21. The complex of claim 18, further complexed with a bone morphogenic protein to transduce an intracellular signal.

22. The complex of claim 21, wherein the bone morphogenic protein is OP-1.

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(57) Abstract

The present invention relates to a novel TGF-β superfamily type II receptor having binding affinity for a bone morphogenic protein, osteogenic protein 1 (OP-1). The novel type II receptor has a serine/threonine kinase domain followed by a long serine and threonine rich region in the C-terminal end. The invention further provides nucleic acids encoding the novel type II receptor as well as vectors and host cells expressing the nucleic acids and recombinant production methods using the nucleic acids. Complexes of the novel type II receptor with an appropriate TGF-β superfamily type I receptor are also provided.

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X,P	PROC. NATL. ACAD. SCI. USA, vol. 92, 1995, pages 7632-7636, XP002007953 B. ROSENZWEIG ET AL.: "Cloning and characterisation of a human type II receptor for bone morphogenetic proteins" see the whole document ---	1-22

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WO-A-9614412	17-05-96	NONE	